REMARKS

As was noted in the Applicants most recent response, Applicants can demonstrate possession of the present invention prior to the March 27, 1998 section 102(e) date of Bryan. The priority date of the present application is February 18, 1999, based on the original Singapore application 9900811-2, whose disclosure it can be seen is very similar to that of the present invention.

Enclosed is a declaration executed by each of the inventors, which clearly demonstrates conception of the invention prior to March 27, 1998 and diligence from a time just prior to that date up until the filing of the priority application on February 18, 1999.

The inventors first note that they are submitting this declaration to demonstrate that they conceived of the claimed invention prior to March, 1998 and exercised reasonable diligence in reducing it to practice from a time just prior to March, 1998 up to the time that their Singapore priority application was filed on February 18, 1999.

The inventors then refer to Exhibit 1, which is an application for research grant dated prior to March, 1998, which lists Drs. Zhiyuan Gong and T. J. Lam as principal investigator and key team member, respectively. They state that as can be seen from the abstract on page 2 of the document, a key aim of the project was to prepare ornamental fish for providing to the ornamental fish export industry having various fluorescence genes such as the jellyfish gene encoding green fluorescent protein (GFP). The proceed to observe that various objectives consistent with those set forth in the patent application are described in the abstract and list of objectives on page 2. On the 7-page portion of the grant proposal identified as "Annex A," and particularly that portion entitled "Programme" beginning on page 3 of Annex A, they provided a detailed description of how this work was going to be carried out. There, the inventors disclose

in some detail our proposed approach to the isolation and identification of zebrafish genes, tissue specific expression, isolation of zebrafish promoters, preparation of transgenic constructs, introduction of the transgenic DNA constructs into zebrafish, characterization of zebrafish promoter by transgenic expression and generation of stable lines of transgenic zebrafish expressing GFP.

The inventors then refer to Exhibit 2, which is said to be a document, also dated prior to March, 1998, which shows that the above research grant was approved and funded.

The inventors then state that it is evident from the foregoing that the inventors had conceived of the idea of preparing transgenic fluorescent fish expressing a fluorescence gene for the purpose of providing such fish to the ornamental fish industry, as well as a method for preparing such fish, prior to March, 1998.

The declaration goes on to state in paragraph 6 that there were many facets of their ornamental fish project, not the least of which was the identification and cloning of various tissue-specific or ubiquitous promoters that could be used to express the GFP in the ornamental fish, and that on August 10, 1998, they submitted a manuscript for publication entitled "Fast Skeletal Muscle-Specific Expression of Zebrafish Myosin Light Chain 2 Gene and Characterization of Its Promoter by Direct Injection into Skeletal Muscle." This manuscript was subsequently published in 1999 (DNA and Cell Biology, 18:85-95; see Exhibit 3). The furtehr state that as can be seen from Exhibit 3, this manuscript describes in some detail the isolation and characterization of the MLC2f promoter and its testing by direct injection into skeletal muscle. They conclude paragraph 6 by noting that this promoter is an aspect of their invention and was used by them to prepare ornamental fluorescent transgenic fish.

In paragraph 7, the inventors continue by noting that the work that is described in the foregoing article as well as that on other promoters that they were characterizing was being conducted by them diligently in our laboratory during the timeframe beginning prior to March, 1998, leading up to the manuscript submission in August, 1998. They refer to Exhibit 4, which is said to be a collection of research notes from one of their laboratory notebooks demonstrating studies carried out from February 25, 1998 ("25/02") through April 2, 1998. They state that these studies reflect their work in characterizing the MLC2f promoter through a deletion approach. The further state that outward PCR was carried out to delete the proximal MEF2 (a muscle transcription activator) binding site internally in the two previously made MLC2f 5' deletion constructs with the CAT (chloramphenicol transferase) reporter gene: 79-bp and 1005-bp. These two deletion-mutation constructs were then tested together with other MLC2f 5' deletion constructs by direct injection into zebrafish muscle and it is demonstrated that the mutated MEF2 site could be compensated by upstream MEF2 sites (Exhibit 3). They then proceed to provide a brief summary of what is shown in Exhibit 4 (reproduced precisely below):

A. PP. 261-256. Generation of MEF2 internal deletion in the 79-bp and 1005-bp MLC2f 5' deletion constructs:

P. 261, notes on setting up ligation to make two MLC2f internal-deletion constructs (79-bp and 1005-bp) after outward PCR and BamHI digestion that facilitated the ligation of compatible DNA ends.

P. 260, PCR reactions to ensure that the ligation was successful. One ligation was successful (79-bp construct) and the other one (1005-bp) is less successful. The 1005-bp was repeated by PCR and the result was improved but the band was still faint.

P. 259, Notes on establishing optimal PCR conditions to select internal deletions from the ligation.

P. 259-258, (March 2, 2008) preparation of CAT cloning vector by removing the MLC2f fragment from the pMLC2f-934CAT construct after HindIII and SpeI digestion.

P. 258, ligation of the 79-bp MLC2f promoter fragment to the CAT vector after internal deletion of the proximal MEF2 binding site (March 2), followed by transformation of the ligation mixture into bacterial cells (March 3).

P. 258-257. screening of bacterial colonies containing of correct DNA clones by PCR (March 4-5).

P. 257. DNA sequencing reaction to confirm the clones (March 6).

P.257-256, Similar cloning and selection of bacterial colonies for the 1005-bp internal deletion construct (March 5-11).

B. P. 255-the end. Measurement of CAT activity after injection of MLC2f deletion constructs into zebrafish muscle:

P. 255. Preparation of all MLC2f-CAT constructs for muscle injection experiments. All constructs were transformed into bacterial cells and monitored by PCR.

P. 254. Large scale of plasmid preparation for MLC2f-CAT constructs and OD reading to monitor quality of plasmid preparations.

P. 253. radioactivity reading printed directly from a scintillation counter to measure CAT activity.

P. 252, blank page.

P. 251, Calculations of CAT activity based on raw data.

P. 250, Summary of relative CAT activities for different CAT constructs at different time points and dosages.

The last page, more raw data printed directly from a scintillation counter in these experiments.

In paragraph 8 of the declaration, the inventors continue by noting that during this time frame they were also attempting to identify other zebrafish genes and promoters that could be used in the preparation of our ornamental transgenic fluorescent fish. One such gene was the ARP gene. They state that Exhibit 5 shows some studies relating to their isolation and characterization of the ARP gene expression and promoter. The first two pages of Exhibit 5, dated March 2-5, 1998, are said to be notes for preparation of ARP probe (cDNA clone A150) for in situ hybridization to characterize ARP expression in zebrafish embryos. The third page shows studies dated March 23, 1998, relating to the cloning of a long ARP promoter (2.1 kb) to the pEGFP-1 vector (Clontech). The next few pages are sequence analyses of the 2.1 kb ARP promoters. The sequencing of the ARP cDNA (A150) and promoter elements, which they state is shown in the remainder of Exhibit 5, were said to be carried out over the next several months, as evidenced by the ultimate sequencing, carried out on August 23, 1998.

In paragraph 9 of the declaration, the inventors refer us to Exhibit 6, which is said to be a collection of pages from their laboratory notebooks that evidence our work on characterization of the MCK promoter during March, 1998. These studies included the following:

P.31. The zebrafish MCK promoter was cloned into the pEGFP-1 vector and concentration of the MCK5-EGFP plasmid was determined by gel electrophoresis (March 3, 1998). In order to analyse the MCK

promoter, 5' nested deletion was carried out and the MCK5-EGFP plasmid was first cut by restriction enzymes (March 13).

P.32-33, ExoIII nuclease digestion was carried out to conduct unidirectional deletions followed by ligation (continued from March 13) and transformation (March 14). PCR was used to identify colonies with suitable deletions (March 16). Suitable colonies were inoculated for plasmid preparation (March 17-18).

P. 34, DNA concentrations of deleted MCK promoter-EGFP constructs were determined by OD reading (March 23). MCK-CAT construct was made and screened (March 25).

PP.34-37. Preparation of a series of plasmid DNAs for functional analyses and these plasmid DNAs included MCK5-EGFP, CMV-EGFP, MLCP1.2-EGFP, ARP0.8-EGFP, MCK5-CAT, MCK23-CAT (MCK5 and MCK23 are two different MCK gene promoters).

The immediate next page, raw data of CAT activities of different CAT constructs after muscle injection.

The following three pages are DNA sequence data of deleted MCK promoters (MCK5-d3, MCK5-d6 and MCK5-d7).

The last two pages are the complete sequence of the MCK promoter with indication of the start sites of deletion constructs (d3, d6. d10. d5, d12, d7 and d9).

In paragraph 10 of the declaration, the inventors refer us to Exhibit 7, which is said to display studies and experimental notes for making 5' deletion constructs in the EGFP vector for

characterization of zebrafish MLC2f and ARP promoters. They state that the approach used here was unidirectional deletion by ExoIII nuclease, followed by ligation, transformation and PCR selection of suitable size of promoter constructs for functional analyses. They state that certain of these studies were carried out from October 7, 1998 through October 16, 1998, as shown on pages 38 through 41 of Exhibit 7 as follows:

PP. 38-39, nested deletion of pMLC2kb-EGFP (Oct 7-10, 1998)

P. 40, PCR screening of colonies containing 5' deletions of MLC2 promoter (Oct 9-10).

P. 41, nested deletion of pARP-EGFP and PCR screening for deletion constructs (Oct 15-16)

The ARP promoter analysis data were published in Ju et al (1999) Dev Genetics 25:158-167. Paper submitted on Feb. 4, 1999 and accepted on March 19, 1999. (see Exhibit 8)

In paragraph 11 of the declaration, the inventors refer to Exhibit 9, which is said to set forth their lab notes evidencing their work on characterization of expression of muscle-specific genes in zebrafish embryos including MLC2f and MCK genes during the time frame of June, 1998 through the end of September, 1998, with some additional studies in December, 1998. They state that this work was ultimately published in the publication of Xu *et al.*, Developmental Dynamics, 219:201-215 (2000), attached as Exhibit 10. They then state that the lab notes can be seen to evidence the following activities:

P. 60 Total RNA isolation from zebrafish embryos at different developmental stages (June 8, 1998)

PP. 61-62 Running of RNA gel (June 10), northern blot of same shown at the top of page 62

P. 62 (bottom) PCR amplification and purification of cDNA inserts from various muscle-specific clones (June 11)

P. 63 Summary of in situ hybridization results, including analysis of expression sequence of muscle genes (June 12)

PP. 64-65 Radioisotope-labeling of two muscle gene probes by the random primer approach, E371 (alpha tropomyosin) and MLC, and northern blot hybridization using the two probes (June 13-14)

P. 66-68 Re-running of RNA gel electrophoresis for northern blot experiments and re-preparation of total RNAs from zebrafish embryos of various stages (June 16-30)

P. 69 PCR amplification of desmin fragment (another muscle specific gene) and synthesis of A228 (fast muscle tropomyosin) and MLC2 probes (July 7)

PP. 70–71 Running of RNA gel for RNA blotting studies; preparation of radioactive probes to be used for probing northern blot (July 9-14)

PP. 72-73 More RNA extractions from staged embryos, running of RNA gel (July 22-23)

PP. 73-76 Preparation of non-radioactive RNA probes (DIG [dioxygenin]-labeled) for in situ hybridization and also performance of northern blot hybridization (July 24-29).

p. 77 Blank.

PP. 78-81 Random primer labeling and performance of northern blot hybridization for more muscle-specific probes, desmin, E465 (parvalbumin), E134 (troponin T) and E371 (alpha tropomyosin); photocopies of some autoradiograms of northern blot hybridization are presented in P. 81 (Aug. 3-13)

P. 82 Sequencing reaction to sequence selected muscle-specific cDNA clones (Aug. 14)

P. 83 More random primer labeling of probes E68 (Myosin heavy chain 1) and A354 (troponin C) (Aug 18)

PP. 84–85 RNA extraction from 8 hour and 10 hour embryos, running of RNA gel, labeling of probes and hybridization (Aug 21-23)

P. 86 Northern blot hybridization for desmin, E68, α-actin and MLC3 (Aug. 25-29)

PP. 87-89 Construction of an α-actin gene specific probe starting from amplification of α-actin 3' untranslated region (3'UTR), to purification, ligation and probe labeling. Simultaneously a MCK probe was also prepared by restriction digestion, purification and labeling. (Sept 2-3)

P. 87. MCK promoter was also tested by injection of MCK-EGFP construct into zebrafish embryo. (Sept 2)

PP. 90-91 Continuation of cloning of the α-actin specific probe and confirmation by PCR, and later preparation of the α-actin 3'UTR plasmid for making DIG-RNA probes for in situ hybridization. (Sept 4-10)

PP. 92-95 Preparation of fluorescent α-tropomyosin probe and DIG-RNA probes from eight muscle-specific genes and performance of both single color and double color in situ hybridization. The last two pages are summary of onset of gene expression of these muscle-specific genes based on the in situ hybridization experiments (Sept 16-21).

PP. 96-97. blank.

PP. 98-99 Summary of northern blot hybridization using these muscle-specific gene probes on different adult tissues (Dec 8-11). Some of the hybridization experiments were performed between Sept 7-11 based on the record.

In paragraph 12 of the declaration, the inventors refer us to Exhibit 11, which is said to be an application to hire a postdoctoral fellow to assist in the ornamental transgenic fish project. They state that this application was submitted in August, 1998 and approved on August 27, 1998. This document is said to be of additional relevance in that it describes the studies that had been carried out to date, as well as those contemplated for the future. They point out , for example, on pages 1-2 of the Exhibit 11 application, under the section entitled "Progress to date" that it is noted that such progress included 1) the isolation of a few hundred zebrafish genes (cDNAs) encoding a wide range of proteins and expressed in a wide variety of tissues, which would provide a rich resource for developmental analysis and isolation of gene promoters, 2) the development of a rapid method to isolate gene promoters, including the fact that six gene promoters had been isolated to date, one from the cytokeratin (CK) gene for skin specificity, three for muscle specificity from a myosin light chain 2 (MLC2) gene and two muscle creatine kinase (MCK) gene, as well as a acidic ribosomal protein P0 (ARP) gene for ubiquitous

expression, 3) demonstrated that the skin specific promoter and muscle specific promoter can direct GFP expression correctly in the respective tissues, and 4) that stable lines of GFP expressing transgenic fish are being developed. This document is also said to be important in that it demonstrates the relevancy of the studies being described in other sections of this declaration (such as the characterization of expression of muscle-specific genes in zebrafish embryos in paragraph 11, above, and Exhibit 8).

In paragraph 13 of the declaration, the inventors refer us to Exhibit 12, which is a summary sheet of DNA injections into zebrafish embryos dated from September 1998 through May 1999, involving the preparation of transgenic embryos for the purpose of testing the activity of various zebrafish promoters. They state that this is a log sheet that their group used to record the dates that the zebrafish embryos were injected, the construct that was injected, the number of embryos that were injected, the number of embryos that survived ("S") and expressed ("E") the GFP at various timepoints post-injection, the tissue specificity of expression and remarks regarding the level of expression. On the first page is recorded 16 separate experiments carried out between September 6, 1998 and February 4, 1999, involving the use of the ARP promoter ("ARP"), the MCK promoter ("MCK"), the MLC (or MLC2, MLC2f and MYLZ2) promoter ("MLC") and the CKP (or CK) promoter ("CKP"). As can be seen, many if not most of these studies resulted in embryos that survived and expressed the GFP at the 48 hour time point. Shown on the second page of Exhibit 13 is a similar log of 5 studies carried out during the month of October, 1998. The last page of Exhibit 13 shows 11 injection studies carried out in May, 1999. During the interim times between injections, the injected embryos were grown up and germline transmission of the transgenes was screened for selection of stable transgenic lines. They were also busy analyzing the data from previous injection experiments and planning further

studies carried out in connection with subsequent injection experiments as well as other relevant experiments such as characterization of muscle-specific expression in zebrafish embryos.

In paragraph 14 of the declaration, the inventors refer us to Exhibit 12, which is said to be a second grant application on transgenic ornamental fish entitled "Production of fluorescent transgenic ornamental fish." The grant application was submitted on February 1, 1999, and funding was requested for additional developmental work on the invention. As can be seen from section II. (i) of the Annex A, this document briefly reviews the work that had been accomplished up until that time on the fluorescent transgenic ornamental fish project. It is stated that transgenic ornamental fish should be more acceptable to regulatory agencies and consumers, and proceeds to outline the approach that the inventors took in the preparation of our ornamental fish, including the use of a gene encoding GFP under the control of a tissue-specific or a ubiquitous promoter (work that is reflected hereinabove). The application then proceeds to note that we had prepared as of that time four GFP transgenic constructs (pCK-EGFP, pMCK-EGFP, pMLC2f-EGFP and pARG-EGFP), and that when these chimeric gene constructs were introduced into fish, all of them showed predictable expression patterns according to the specificities of the promoters used. It is then stated that a patent for the constructs was being filed. (The foregoing is described in the second page of Annex A of Exhibit 12). In the paragraph bridging the second and third pages of Annex A, it is further stated that we intended to isolate additional gene promoters that would permit targeting transgene expression in any tissue, and that we contemplated extending our work in zebrafish to other ornamental fish such as medaka, goldfish, koi, carp and glass catfish. At the bottom of the third page and top of page 4 of Annex A, many additional aspects of the present invention are explained, including, for example, the development of skin-specific, muscle-specific and ubiquitously expressing fish, and

the use of other colors and mixture of colors. Further pages of Annex A include additional details regarding the preparation of other types of transgenic ornamental fish.

In paragraph 15 of the declaration, the inventors conclude and summarize from the foregoing evidence, that it is quite clear that they had conceived of their invention prior to March, 1998 and were diligent in reducing the present invention to practice during the time frame of just prior to March, 1998 through their Singapore filing date of February 18, 1999. They then present the following table, which is a brief summary of their activities during this period:

Period	Activities	Remarks
Prior to March, 1998 (Conception)	Applied for and obtained our first research grant from National University of Singapore to support transgenic ornamental fish research and the title of the project is "Generation of novel varieties of ornamental fish by transgenic expression of green fluorescent protein (GFP)". This document demonstrates both that we had the idea and had a detailed understanding on how to carry it out.	Exhibit 1, 2

March 1998 to April 2, 1998	Characterization of zebrafish MLC2f promoter by direct injection of deletion constructs into zebrafish muscle	Exhibits 3 and 4
March 2, 1998 to August 23, 1998	Characterization of zebrafish ARP gene expression and promoter	Exhibit 5
March, 1998	Characterization of zebrafish muscle-specific MCK promoter	Exhibit 6
Aug. 1998	Preparation and submission of grant proposal to support a postdoctoral fellow to work on the fluorescent transgenic ornamental fish project. The grant was approved on Aug. 27, 1998	Exhibit 11
Oct 7-16, 1998	Preparation of 5' deletion constructs for zebrafish MLC2f and ARP promoters.	Exhibit 7
June 8, 1998 – September 21, 1998, and December, 1998	Characterization of expression of muscle-specific genes in zebrafish embryos including	Exhibits 9 and 10

	MLC2F and MCK genes	
Sept 6, 1998-Feb 4, 1999	Microinjection of zebrafish promoter-GFP constructs	Exhibit 12
Jan 1999	Preparation and submission of the second research grant application on production of transgenic ornamental fish. The grant was submitted to National University of Singapore and entitled "Production of fluorescent transgenic ornamental fish (submitted on Feb. 1, 1999)	Exhibit 13

From the foregoing evidence, Applicants contend that the Bryan patent is not available as prior art.

CONCLUSION

Applicants believe that the foregoing remarks fully respond to all outstanding matters for this application. Applicants respectfully request that the rejections of all claims be withdrawn so they may pass to issuance.

The Examiner is invited to contact the undersigned attorney at (512) 536-3055 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

David L. Parker
Reg. No. 32,165
Attorney for Applicant

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 474-5201
(512) 536-4598 (facsimile)

Date: February 20, 2008